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MOLECULAR, PHYSIOLOGICAL AND MORPHOLOGICAL MARKERS ASSISTED SELECTION FOR SALT-TOLERANT IN SOME EGYPTIAN FABA BEAN VARIETIES

MOHAMED H. ABDELFATTAH[1], REHAB EL HAWARY[1], FATMAH A. SAFHI[2], AMINA ZEDAN[3*] AND MEDHAT E. ELDENARY[1]

[1]*Genetic Department, Faculty of Agricultural, University of Tanta, Tanta, Egypt.*
[2]*Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia*
[3]*Department of Biological and Environmental Sciences, Faculty of Home Economics, Al-Azhar University, Tanta 31732, Egypt*
[*]*Corresponding author's email: AminaZedan1948.el@azhar.edu.eg*

Abstract

Salinity stress is one of the most serious abiotic factors limiting faba bean productivity. The faba bean is an important legume crop because it can give a high yield, and its grains are full of nutrients. This study aims to select by several markers the best variety that adapts to salt stress. Three varieties of *Vicia faba* (Nubaria-1, Nubaria-3, and Nubaria-4) were used in this study. Plant growth parameters, cell membrane stability, photosynthesis pigments and protein profile analysis were measured as indices for salinity responses. As a molecular marker, this study used ten ISSR primers to assess the genetic diversity of three varieties. Conditions of control and salinity stress were used for all determinations. The results indicated that the Nubaria-3 variety had the most effective traits regarding root characteristics and the ability to develop well under salt conditions compared to the other varieties. Protein-profile changes increased in band numbers and intensity, and its expression continued under salinity conditions as appeared with Nubaria-3 and Nubaria-4. Polymorphism and phylogenetic relationship among varieties were investigated by ISSR molecular markers and 22.43% polymorphism was detected, with the highest percentage of genetic similarity between Nubaria-3 and Nubaria-4 reached 73%. As a result, Nubaria-3 could be chosen as a tolerant variety in a subsequent investigation to explore the most essential genes associated with salt tolerance compared to the sensitive variety.

Key words: Faba bean; ISSR marker; Salinity stress; Membrane stability; Polymorphism; Phylogenetic.

Introduction

One of the main abiotic stresses that inhibits plant growth and development is salinity. It is the biggest abiotic risks to plant survival, spread, and productivity is soil-salinity stress (Hussein *et al*., 2017). Salt stress induces metabolic changes, such as a loss of pigment production, a reduction in photosynthetic rate, and an rise in photorespiration rate, that causes a rise in reactive oxygen species generation (ROS) (Arif *et al*., 2020). A nutritional imbalance in plants is another effect of salt stress (Moussa & Hassan, 2016). Many physiological and cellular functions are severely harmed by salt stress, including water absorption, nutrient uptake, plant development, and cellular metabolism, all of which reduce yield (Wang *et al*., 2017). Salt stress induced a noticeable reduction in relative water content while a remarked increase in antioxidant enzymes in leaves with increasing salt concentration (Zayed *et al*., 2017).

Faba bean (*Vicia faba* L.) is well recognized as a prominent legume crop globally, serving as a significant protein reservoir for both human and animal dietary requirements because of its nutritional value, abundant in proteins, carbs, and minerals (Dhull *et al*., 2022).

Faba beans grow in a large range of latitudes (about 40°S to 50°N) and at elevations between 3,000m and sea level (Gnanasambandam *et al*., 2012). 4.46 million tons of dry grains were produced in 2016 (Anon., 2018). Faba beans are widely recognized as the initial leguminous crop cultivated in Egypt. Statistics show that Egypt's faba bean fields have shrunk from about 2715 000 acres in 2000 to about 175.4 000 acres in 2019. In Egypt, there is a trend to grow crops, like faba beans, on newly reclaimed land. However, most of these soils are affected by salinity, a world problem that needs to be fixed quickly. Our study aimed to find different faba bean genotypes that can grow in the newly reclaimed salty grounds. Only a few variants with enhanced tolerance have been developed. To identify the best variety that adapts to salt for that, In this study, we tested the different responses of three Egyptian varieties of faba beans under various levels of salinity stress on several morphological, physiological, biochemical and molecular characteristics.

Materials and Method

Plants and growth conditions: Three varieties of Vicia faba (Nubaria-1, Nubaria-3 and Nubaria-4) were received from the Agricultural Research Centre, Kafr El-Sheikh, Egypt. This experiment was carried out in the Faculty of Agriculture at Tanta University farm during the 2020 - 2021 winter seasons. Seeds were placed in pots containing about 5 Kg of soil and divided into three groups with three replicates for each variety. First group is the control treatment, where pots were rinsed with tap water. Second group, pots were irrigated with 100 mM NaCl solution as level-1 of salinity. Third group pots were irrigated with 200 mM NaCl solution as level 2 of salinity treatments. Samples were collected for growth measurements after 40 days of planting and some of them were stored at -80ºC for further analysis.

Determinations of plant growth parameters: A ruler was used to measure the shoot and root lengths in centimetres, each variety's branches were counted, and plants were photographed. Shoots were removed from roots to weigh fresh and dry. After gently washing each plant with distilled water and drying with a paper towel,

the fresh weights (FW) were calculated. Reweighing was done to ascertain the dry weight (DW) following three hours of oven drying at 105°C.

Estimation of photosynthetic pigments: Half a gram of leaf tissue was extracted with 80% acetone to measure the levels of total chlorophyll (Chll), chlorophyll a, chlorophyll b, and carotenoids According to Arnon (1949) approach; a UV1901PC spectrophotometer was used to analyze the pigment at 645, 663, and 470 nm. The blank was made up of 80% acetone. The following are the equations:

$$\text{Chll a } \left(\frac{\text{mg}}{\text{mL}}\right) = 0.0127 \times \text{A663} - 0.0027 \times \text{A645}$$

$$\text{Chll b } \left(\frac{\text{mg}}{\text{mL}}\right) = 0.0229 \times \text{A645} - 0.0046 \times \text{A663}$$

Total Chll (mg/mL) = Chlorophyll a + Chlorophyll b

$$\text{Carotenoids } \left(\frac{\text{mg}}{\text{mL}}\right) = \text{A470} + (0.1140 \times \text{A663}) - 0.6380 \times \text{A645}$$

Membrane stability: Electrolyte leakage (EL) was measured for each varieties using a conductivity meters (AdwaAD32). Leaf disks (0.5 g) were placed in a tube with 20 mL of de-ionized water. The solution's conductivity was assessed after the vials were softly shaken. After an hour, the solution's conductivity was once more tested. Finally, each vial was put in a pot of boiling water for one hour. The total conductivity was determined after letting the vials cool to room temperature. According to Omar *et al*., (Omar *et al*., 2012), the electrolyte leakage ratio was estimated as the solution's net conductivity with leaves immersed for one hour, split by the final conductivity total upon boiling. The result is expressed as $\mu Scm^{-1}*FWh^{-1}$.

Determination of total protein content: One half gram of plant tissue was pulverized in liquid nitrogen to a very fine powder. Homogenizing the powder in 1.0 millilitre of cold extraction buffer was done on ice (2% SDS, 100 mM Tris HCl (pH 8) 2-mercaptoethanol (10 mM) and 5 mM NaCl). The bovine serum albumin was the reference, the concentration of the isolated proteins was assessed following the Bradford methodology (Bradford, 1976). According to Laemmli (Laemmli, 1970), SDS-PAGE was accomplished using a discontinuous buffer system. Before loading onto the gel, the protein samples were denatured in SDS (1x) gel loading buffer by heating for 5 min at 95°C. Samples with standardized protein amounts (15g) were loaded into the gel. Pre-stained molecular protein ladder (Gene Direx's BLUeye) was utilized.

DNA extraction: For genomic DNA isolation, leaves from plants that were one month old were employed. The plant's EZ-10 Spin Column Genomic DNA Minipreps Kit was used to extract genomic DNA, which was then treated with RNase-A. On a 0.8% agarose gel, the extracted DNA's quantity and quality were evaluated. The extracted DNA was used for polymorphism analysis using sets of chosen primers.

Inter simple sequence repeats (ISSR) polymorphism analysis: A set of ten primers (Table 1) were used for ISSR-PCR. The selected primers were recruited in PCR amplification reactions following instructions supporting MyTaqTM Red Mix, 2x. BIOLINE. Amplification was programmed for a 5-min initial denaturation step at 94ºC followed by 35 cycles of denaturing at 94ºC for one minute, annealing at 38-52ºC (according to each primer) for one minute, extension step at 72ºC for one minute and a final extension step at 72ºC for 5 min by using (MyGene®–MG96G) programmable thermal cycler. The amplification products were electrophoresed against a DNA ladder (250 bp and 10000 bp) to estimate the molecular sizes of the amplified fragments.

Analyzing data and creating phylogenetic trees: Using the PAST application, version 1.90, separated bands were assessed and scored depending on the absence and presence of bands (0 and 1 respectively). Using Past software (version 2.17) created by (Hammer & Harper, 2001), The unweighted pair group technique of arithmetic averages (UPGMA) was used to find the genetic similarity (based on Jaccord's formula) between the tested varieties (Sneath & Sokal, 1973).

Table 1. The recruited set of ISSR primers for detecting polymorphism among three *Vicia faba* varieties.

Primer name	Number of nucleotides	Sequence (5` to 3`)	Annealing temperature (ºC)
ISSR. 5	11	GTGGTGGTGGC	38
ISSR. 15	11	CACCACCACGC	38
ISSR. 16	11	GAGGAGGAGGC	38
ISSR. 14	11	GTGGTGGTGCG	38
ISSR. 157	17	AGCAGCAGCAGCAGCGA	51
ISSR. 158	17	AGCAGCAGCAGCAGCGT	51
ISSR. 160	17	AGCAGCAGCAGCAGCGC	52
ISSR. 161	16	CACACACACACACAAC	43
ISSR. 162	16	CACACACACACACAAT	41
ISSR. 163	16	CACACACACACACAGA	43

Statistical analysis

This study used a full factorial split-plot design with randomised complete blocks for all of its trials with salinity as main plots and varieties as subplots. All experiments used at least three replicates for each treatment. ANOVA was used to test for significant differences between salinity levels (pSalinity), varieties (pvarieties), and their interaction (pSalinity × pvarieties). Tukey's significant difference test was used for post-hoc analysis (pSalinity × pvarieties 0.05).

Results

Growth parameters

Shoot and root length: Changes in shoot and root growth were observed by changes in shoot length and root length and their spread for three varieties of faba bean under control and two levels of salinity stress conditions. Photos of whole plants illustrate the change in roots and shoots shown in (Fig. 1). Salinity stress caused growth inhibition of plants by reducing the shoot length; all varieties showed a significantly dramatically decreased shoot length under salinity stress compared to control (Fig. 2A). Nubaria-3 variety showed the highest value of shoot length under control and salinity stress conditions, while Nubaria-1 was the variety most affected by salinity stress. Under salinity stress series changes in root lengths among the studied varieties were observed (Fig. 2B). In contrast to shoot, there was an increase in root with an increase in salinity stress in both Nubaria-3 and Nubaria-4 varieties; they showed a significant increase in root length under salinity stress compared to control. While the Nubaria-1 variety showed a non-significant decrease in root length under salinity stress compared to the control.

The fresh and dry shoot's weights: The results obtained (Fig. 3) showed variations in the shoot fresh weights (Fig. 3A) and dry weights (Fig. 3B). Under control conditions, Nubari-1 showed the highest value of FW and DW compared with all varieties. In this study, Nubaria-1 showed higher values in FW and DW compared with the other varieties under 100 mM NaCl as level-1 of salinity stress. While with increased salinity stress level to 200 mM NaCl, both Nubaria-1 and Nubaria-4 showed a noticed decrease in DW and FW compared to Nubaria-3.

Root fresh (FW) and dry weights (DW): In this study, all varieties display a noticeable increase in root DW and FW under salinity stress conditions compared to the control (Figs. 4A and 4B). The Nubaria-3 variety recorded the highest values in DW and FW under salinity stress conditions compared to the other varieties in this study. With increased salinity stress to 200 mM NaCl, Nubaria-1 and Nubaria-4 decrease in DW and FW compared to 100 mM NaCl.

Changes in the branches number: The number of plant branches was significantly reduced by salinity. In this study, all varieties significantly reduced the number of plant branches per plant with salinity stress compared to the control (Fig. 5). Nubaria-1 variety is considered to have a high number of branches per plant; its record is the highest in branches compared to other varieties in the control condition. The Nubari-4 variety showed the lowest number of branches under salinity stress, 200 mM NaCl, compared with control and other varieties.

Changes in photosynthesis pigments: Changes in the total chlorophyll and carotenoids for all varieties under salinity stress and control conditions were shown in (Figs. 6A and B). The highest total chlorophyll and carotenoid value was recorded under control in the Nubaria-3 variety. Salinity stress showed a significant drop in the quantity of chlorophyll in all varieties, and the chlorophyll inhibitions increased with the increase of salt stress 200 mM NaCl. Also, the Nubaria-3 variety showed a non-significant increase compared with other varieties.

On the other hand, carotenoid content showed a non-significant decrease under 100 mM NaCl in all varieties. Still, both Nubaria-1 and Nubaria-4 showed a significant decrease under 200 mM NaCl compared to control. Also, Nubaria-3 recorded the highest value of carotenoids under stress conditions compared with other varieties.

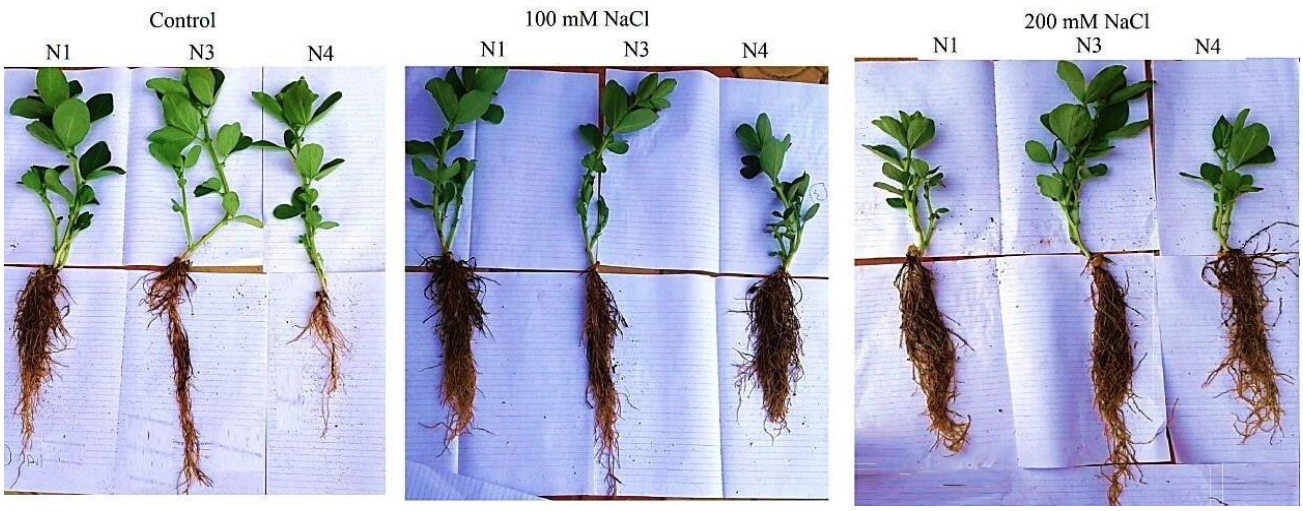

Fig. 1. Changes in root and shoot lengths of three varieties N1 (Nubaria-1), N3 (Nubaria-3), and N4 (Nubaria-4), under untreated conditions and stress of salinity.

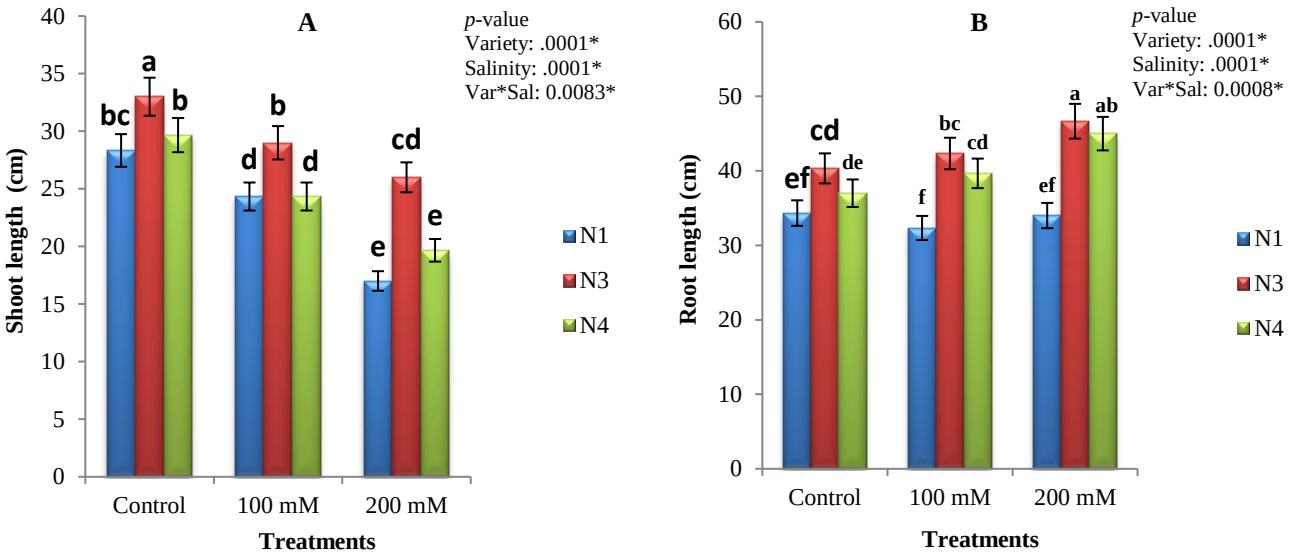

Fig. 2. Changes in shoot lengths [A] and root length [B] of all varieties under untreated conditions and stress of salinity.

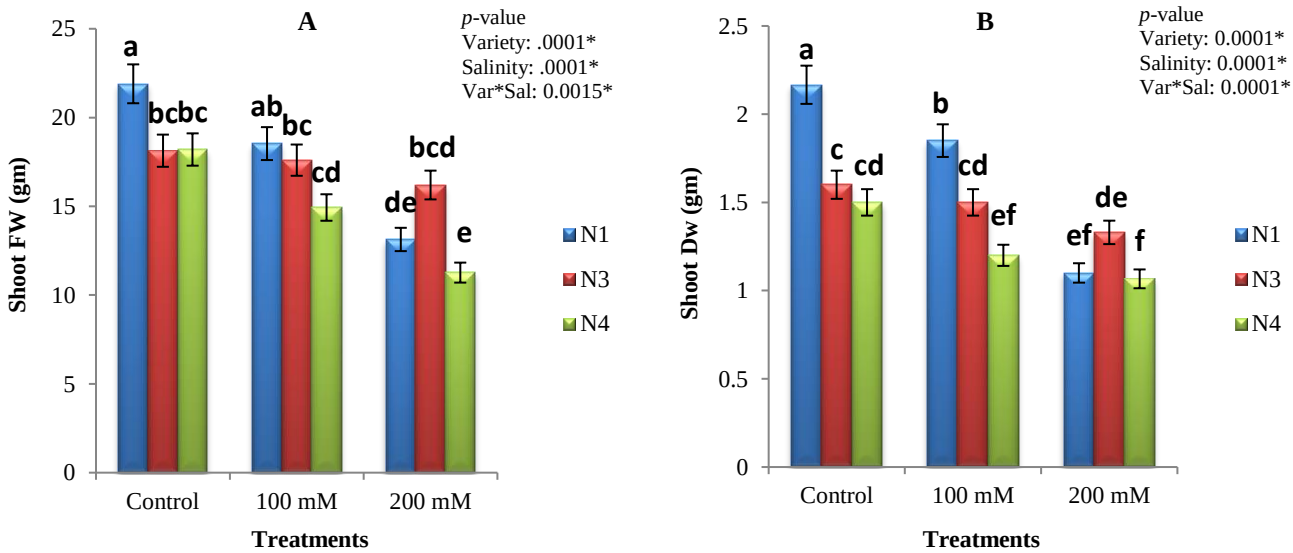

Fig. 3. Changes in shoot fresh weight (FW) [A] and dry weight (DW) [B] of all varieties under control and salinity stress conditions.

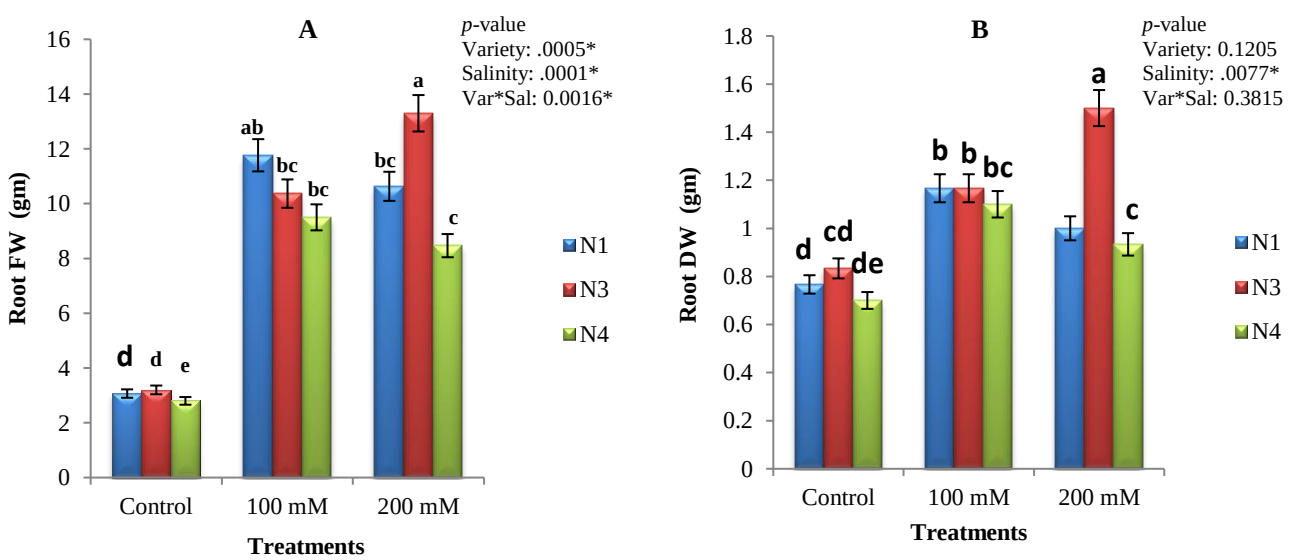

Fig. 4. Alteration in root fresh weights (FW) [A] and root dry weights (DW) [B] of all varieties under control conditions and salinity stress.

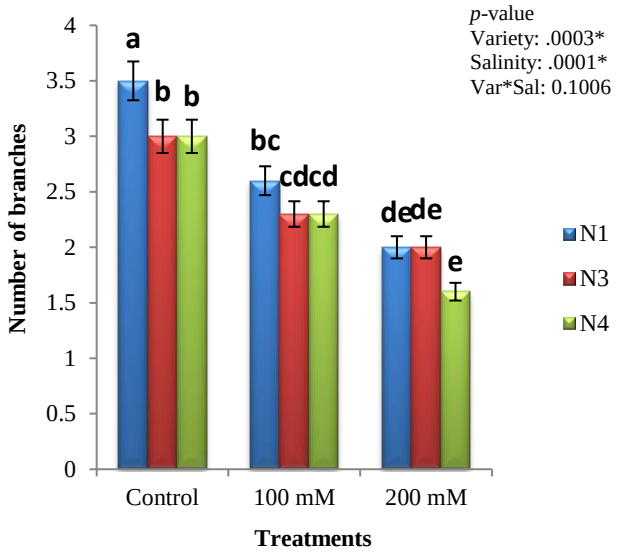

Fig. 5. Changes in the number of branches per plant for all studied varieties under control and salinity stress conditions.

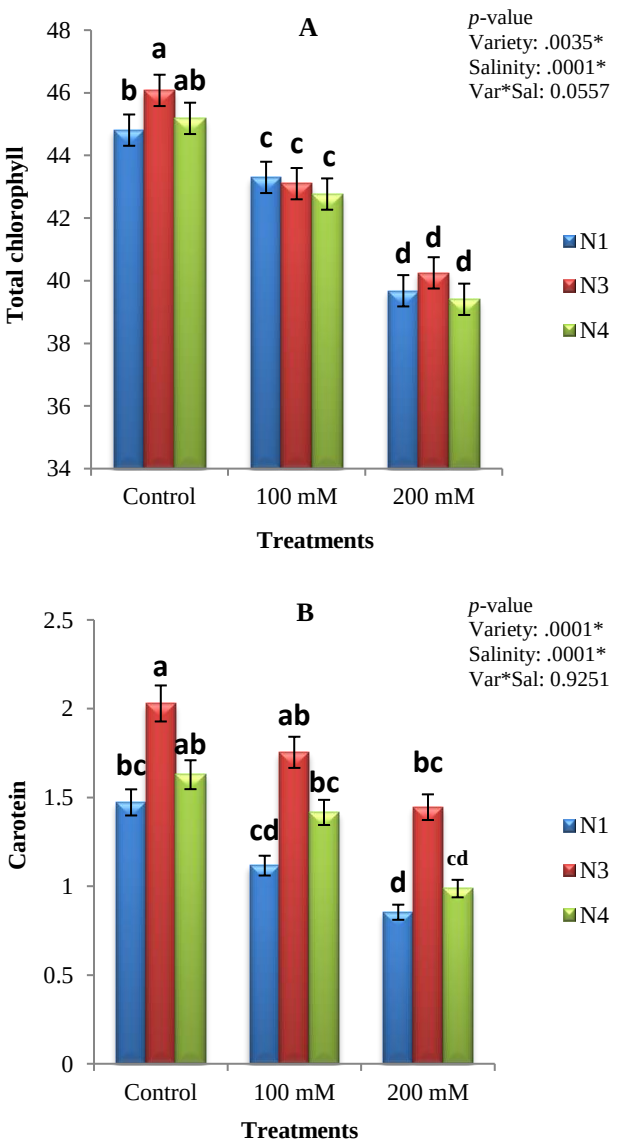

Fig. 6. Changes in the total chlorophyll [A] and carotenoids [B] of all varieties under control and salinity stress conditions.

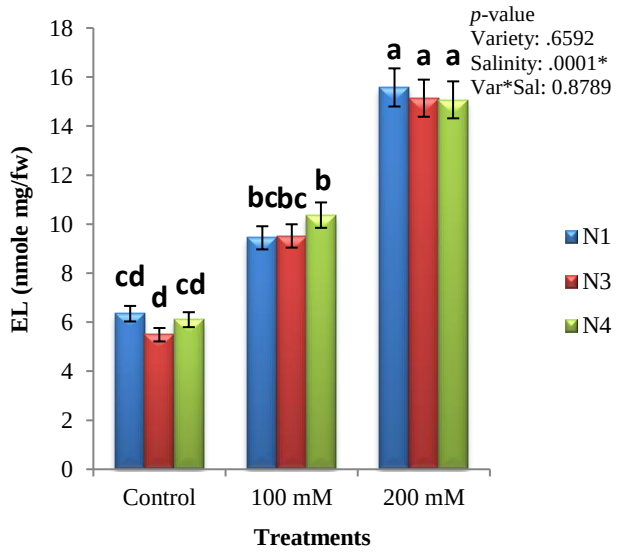

Fig. 7. Changes in electrolyte leakage (EL) of all varieties under control and salinity stress conditions.

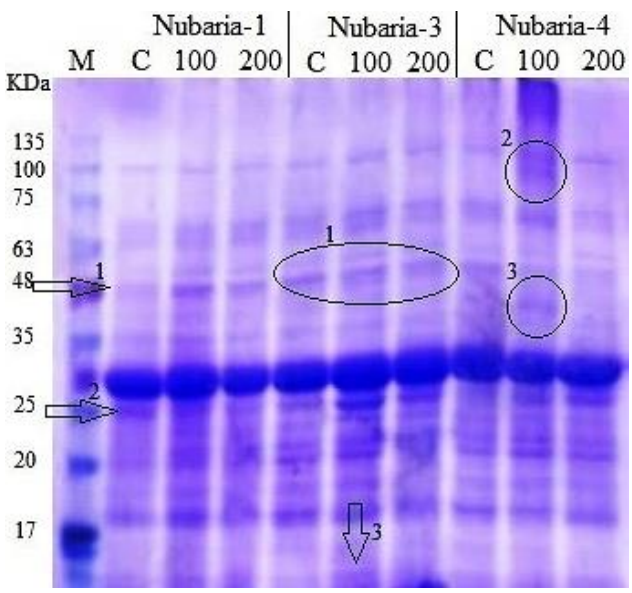

Fig. 8. Total protein isolated from all types under salt stress and control conditions was analyzed using SDS-PAGE.

Changes in membrane stability: The electrolyte leakage (EL) rate as evaluated by changes in membrane stability, as illustrated in (Fig. 7). In all varieties, salinity stress led to a considerable rise in EL values compared to control. Under salinity stress, data showed non-significant variations in EL values between varieties.

Analysis of total protein: Under experimental conditions, electrophoresis examination of total protein fractions from leaves of all varieties revealed many alterations in protein patterns (Fig. 8). These modifications included enhanced expression of some protein bands under salt stress as measured by increased band volume and darkness compared to control, such as protein bands indicated by arrow1 with molecular weight ~ 48 kDa under 100 mM NaCl treatment. In contrast, this band decreased at the salinity level of 200 mM NaCl

treatment in Nubaria-1 and Nubaria-4. However, the expression of this band was continuous under control and salinity conditions with Nubaria-3. Other bands appeared with both Nubaria-3 and Nubaria-4 under stress, while this band was absent in Nubaria-1, indicated by arrow2 with molecular weight ~ 25 kDa. The expression of new protein bands was another shift in protein pattern (circular zones 2 and 3) with Nubaria-4 and arrow3 with Nubaria-3 under stress conditions.

Inter simple sequence repeats polymorphism (ISSR) marker: The electrophoretic pattern of the amplified PCR- products yielded by ISSR for faba bean varieties is shown in (Fig. 9). Ten ISSR primers in all were employed to evaluate the genetic diversity of three varieties in this study. Table 2 displays the total number of amplified, monomorphic, and polymorphic fragments and the percentage of polymorphism found for each ISSR primer. Among the 10 ISSR primers tested, only 12 produced bands were polymorphic among varieties. An average of 6.1 bands per primer was amplified (ranging from approximately 150 to 4000 bp), and 22.43% were polymorphic. The primer157 and primer161 presented the highest percentage of ISSR polymorphism (50%). In Nubaria-1, primer 15 presented four unique bands; primer 14, 158, 160, and 161 presented one unique band. While primer 16, 158, 161, and 163 presented one unique band in the Nubaria-3 variety. Both primers 14, primer157, and primer158 showed one unique band in Nubari-4 (Fig. 9).

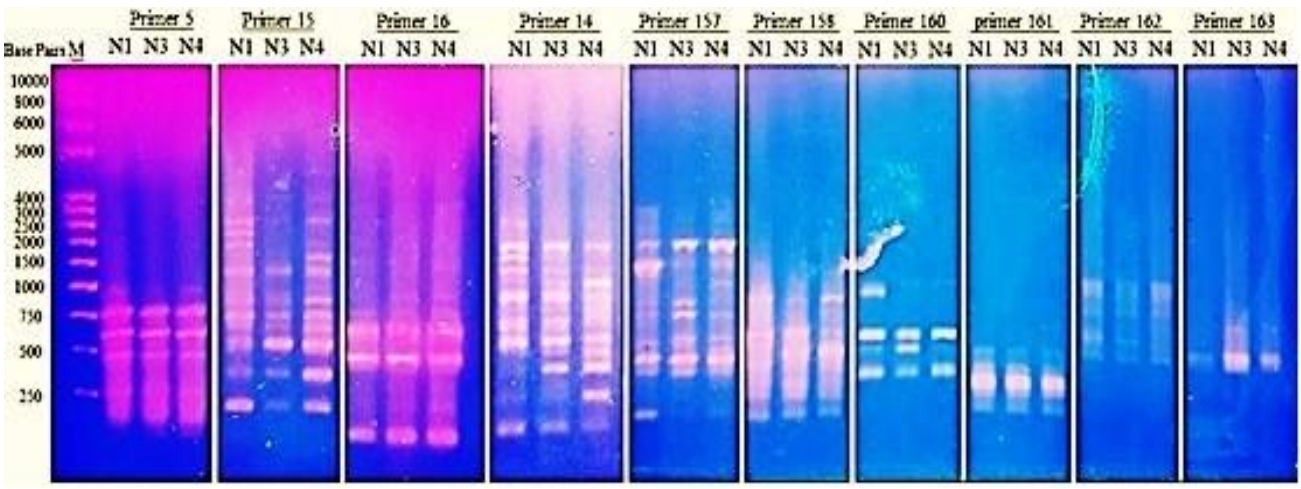

Fig. 9. DNA profiles of three faba bean varieties as screened by ten ISSR primers.

Table 2. The number of fragments (monomorphic or polymorphic fragments), also, the polymorphism percentage that obtained by ISSR primers for three bean varieties.

Primers	Range of fragments size (bp)	Total Nou. of fragments	Fragments of monomorphic	Fragments of (polymorphic)	Unique fragments	Polymorphism %
primer5	200-1000	5	4	1	0	20
primer15	200-4000	13	7	1	5	7.69
primer16	150-750	3	2	0	1	0
primer14	150-2500	10	5	1	4	10
primer157	200-3000	4	1	2	1	50
primer158	200-900	10	5	2	3	20
primer160	350-1000	6	3	2	1	33.33
primer161	200-500	4	0	2	2	50
primer162	500-1000	3	3	0	0	0
primer163	500-650	3	1	1	1	33.33
Total		61	31	12	18	
average		6.1	3.1	1.2	1.8	22.43

Table 3. Similarity and distance indices among three varieties according to ISSR pattern, using ten primers.

	Nubaria-1	Nubaria-3	Nubaria-4
Nubaria-1	1	0.61	0.67
Nubaria-3		1	0.73
Nubaria-4			1

According to band polymorphisms produced by ISSR-PCR employing the primers, the genetic distances dendrogram among the three tested types is displayed in (Fig. 10). The dendrogram separated three varieties into two clusters. The first cluster included Nubaria-1, and the second cluster included Nubaria-3 and Nubaria-4. The genetic similarity between Nubaria-1 and Nubaria-3 was

61%, while the percentage of genetic similarity between Nubaria-1 and Nubaria-4 was 67%. The highest percentage of genetic similarity showed between Nubaria-3 and Nubaria-4 was 73% (Table 3).

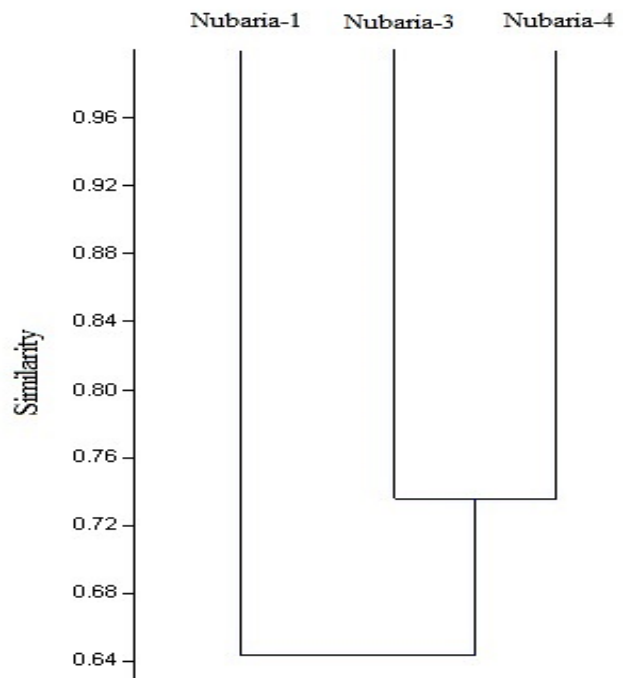

Fig. 10. UPGMA phylogenetic dendrogram, using ten primers, represents the genetic distance for three faba bean varieties according to ISSR pattern.

Discussion

Salinity stress caused growth inhibition of plants by reducing the shoot length; all varieties showed a significantly dramatically decreased the shoot lengths under salinity stress condition compared to control. The nubaria-3 variety showed the highest value of shoot length with control and salinity stress conditions. In contrast, to shoot, there was an increase in root with an increase in salinity stress in both Nubaria-3 and Nubaria-4 varieties; they showed a noteworthy rise in root length under salinity stress conditions in comparison to control. Kumar *et al*. (Kumar *et al*., 2021) concluded that, as the NaCl contents increased, salt stress drastically reduced the plant's total height, shoot and root lengths, and the number of leaves and branches. Also, salinity stress promoted limitations in several physiological attributes (El-Dakak *et al*., 2023). Salts in the soil can draw water. Less water, as a result, is available for the seedlings to absorb, producing root dehydration and water stress. Physiological drought is what this is; if left untreated, it can stop plants from growing as much (Menezes *et al*., 2017). Since roots are in direct contact with salt ions and are important for nutrient and water uptake, an increase in the shoot-to-root ratio is attributed to salt tolerance (Maggio *et al*., 2007).

In this study, Nubaria-1 showed high values compared with the other varieties in shoot DW and FW under 100 mM NaCl as level-1 of salinity stress. While with increased salinity stress level to 200 mM NaCl, Nubaria-3 significantly increased shoot DW and FW compared to the other varieties. Under salinity stress, some plants tend to increase the spread and lengths of roots. In this study, all varieties showed a significant increase in root F.W and D.W under salinity stress compared to the control. The Nubaria-3 variety recorded the highest value in F.W and D.W under salinity stress compared to other varieties in this study.

Previous research on many plants demonstrated that during NaCl stress, the fresh and dry weights of the root and shoot decreased (Kapoor & Pande, 2015; Huang *et al*., 2024). Meriem *et al*.(Meriem *et al*., 2014) reported that under various NaCl treatments, sensitive plants showed a greater drop in biomass than tolerant plants. Salinity raises osmotic stress, this stops water from being taken in and moved. A sequence of hormone-induced processes are set off by this inhibition, and they have the potential to reduce the rate of CO_2 absorption, stomatal opening, and photosynthetic activity (Sarker & Oba, 2020). An additional factor that may be contributing to the slowing in growth is the redirection of energy away from growth and towards the maintenance of salt stress homeostasis, as well as a reduction in carbon gains (Sarker & Oba, 2020). In this study, the Nubaria-3 variety performed most effectively regarding root characteristics and the ability to develop well under salt conditions compared to the other varieties.

Chlorophyll and carotenoids' primary role in photosynthesis is light absorption to ignite electrons inside the pigments. These set off a series of reactions that enable photosynthesis to convert carbon dioxide and water molecules into glucose. The highest total chlorophyll and carotenoid value was recorded under control in the Nubaria-3 variety. Salinity stress showed a significant drop for the chlorophyll in all varieties, and the chlorophyll inhibitions increased with the increase of salt stress (NaCl 200 mM). However, on the other hand, carotenoid content showed a non-significant decrease under 100 mM NaCl in all varieties. Nubaria-3 recorded the highest value of carotenoids under stress conditions compared with other varieties. Salinity is a stress condition that affects photosynthesis at all stages. Damage caused by stress factors at any concentration may decrease a plant's total capability for photosynthetic activity since photosynthesis requires multiple components (Ashraf & Harris, 2013). Stomata close due to saline exposure, limiting photosynthesis (Hniličková *et al*., 2017). In addition, the activity of a number of stomatal enzymes that are involved in the reduction of carbon dioxide (CO2) might have a detrimental influence on the osmotic effects that are generated by salt (Rasouli *et al*., 2021). Salinity's effects on photosynthesis could include preventing electron transport and deactivating the PSII reaction centres (Pan *et al*., 2021). Carotenoids safeguard the photosynthetic system from too much light and act as auxiliary light-collecting pigments. They are crucial parts of the photosynthetic antenna and complexes of reaction centre and are also in charge of colouration, antioxidant defence, and many functions in various plant tissues (Maslova *et al*., 2021). Although increased the concentration of carotenoid in response to salinity stress is a cultivar-specific feature, the majority of salt-resistant cultivars have a higher carotenoid synthesis (Leiva-Ampuero *et al*., 2020).

The electrolyte leakage (EL) measurement ratio calculates the relative membrane permeability or index of membrane stability. In all varieties, salinity stress led to a considerable rise in EL values compared to control. Under salinity stress, data showed non-significant variations in EL values between varieties. Salt stress harms plants by increasing photorespiration rate, leading to increased reactive oxygen species (ROS) production (Zhang *et al*., 2016). The high production in electrolyte leakage due to salinity has always been employed in prior investigations as a sign of enhanced plasma membrane permeability. It is crucial to note that the proteins of membrane transport (carriers and channels) and membrane lipid components allow ions to escape from the leaf discs. Therefore, it is hypothesized that altered proteins and lipids in both membrane components and enhanced plasma membrane permeability are caused by salt stress (Daneshmand *et al*., 2010; Tiwari *et al*., 2010).

Under experimental conditions, electrophoresis examination of total protein fractions from leaves of all varieties revealed many alterations in protein patterns. These modifications included enhanced expression of some protein bands under salt stress as measured by increased band volume and darkness compared to control. In contrast, this band decreased at the salinity level of 200 mM NaCl treatment in Nubaria-1 and Nubaria-4. However the expression of this band was continuous under control and salinity conditions with Nubaria-3. Other bands appeared with both Nubaria-3 and Nubaria-4 under stress, while this band was absent in Nubaria-1. According to reports, soluble proteins cause certain plants' salinity to decrease while increasing salinity in others (Li *et al*., 2015).

Additionally, salinity prevents most shoot proteins from being produced (Khan *et al*., 2024). In reaction to the stress of salt, many proteins accumulate in plants. Normally, under salt stress, plant tissues react by either destroying proteins or generating a lot of proteins related to salt stress (Wang *et al*., 2015). Furthermore, it has been shown that salt-tolerant cultivars of several crops contain more proteins than salt-sensitive cultivars. The total amount of soluble proteins may therefore not be as crucial for salt tolerance as protein quality and type (Dissanayake *et al*., 2022). The critical metabolic functions that these proteins support have a direct role in determining novel varieties that can adapt to salt-stressed conditions. Therefore, the contribution of particular proteins to salt tolerance mechanisms is more significant than the total amount of proteins.

A total of 10 ISSR primers were used to determine the diversity of genetic of three varieties in this study. As a result, the study's findings on the discrimination capability of ISSR markers imply that they might be utilized to precisely and effectively assess the diversity of faba bean genotypes and promote targeted crossover techniques. Molecular markers are unaffected by environmental circumstances, may be easily adopted, and are safe for choosing exceptional agricultural features. Several DNA markers, including inter-simple sequence repeats (ISSR), have been discovered and successfully utilized to define genetic variation in many crop plants. Previous studies have achieved outstanding success in identifying genetic similarities or variances in faba bean breeding programs (Alghamdi *et al*., 2011). Inter-simple sequence repeat markers were also used to document and examine the genetic diversity of the faba bean. (Asfaw *et al*., 2018). It was discovered that the ISSR markers were useful for determining the variety amongst the bean accessions and permitting the adoption of certain contrasting accessions in genetic refinement programmes to meet producer demands (Salazar-Laureles *et al*., 2015).

The future aspect of this study is to select salinity resistant variety and compare with sensitive variety to select the molecular marker for salinity resistance.

Conclusions

Salinity affects all growth and physiological parameters depending on the *Vicia faba* variety. The growth and physiological parameters including shoot and root length, shoot and root fresh and dry weight, total chlorophyll and carotenoids, as well as the profile of protein and DNA in comparison with control plants. This response differs with variety. The varieties of *Vicia faba* can be tolerant to many stresses, including salinity. Using DNA marker and protein profile side by side with morphological and physiological parameters, we select the most variety more tolerant to salinity, Nobaria 3. Nubaria-3 variety performed most effectively regarding root characteristics and the ability to develop well under salt conditions compared to the other varieties.

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